1997

Serotonin Production and Neuron Proliferation in *Drosophila melanogaster* in Two Environments

Cynthia Forsman-Earl
College of Saint Benedict/Saint John's University

Serotonin Production And Neuron Proliferation in *Drosophila melanogaster* in Two Different Environments

A Thesis

College of St. Benedict / St. John's University

In Partial Fulfillment
of the Requirements for the Distinction "All College Honors"
and the Degree Bachelor of Arts

In the Department of Biology
by
Cynthia Forsman-Earl
May, 1997

Project Title: Serotonin Production and Neuron Proliferation in *Drosophila melanogaster* in Two Environments

Approved by:

Dr. Marilyn Meinke, Assistant Professor, Department of Biology

Dr. Charles Rodell, Professor, Department of Biology

Dr. Elizabeth Wurdak, Associate Professor, Department of Biology

Dr. James Poff, Chair, Department of Biology

Director, Honors Thesis Program

Director, Honors Program

Serotonin Production and Neuron Proliferation in *Drosophila melanogaster* in Two Different Environments

Cynthia Forsman-Earl, College of St. Benedict, Biology Department

ABSTRACT

Environmental stimulus is suspected to play a role in both serotonin levels and neuron proliferation in the brain. To understand the relationship between environmental stimulus and serotonin levels *Drosophila melanogaster* were raised in two different environments, a stimulus rich and a stimulus deprived environment. The heads were isolated at different times and assayed for serotonin using an ELISA. The heads were also microscopically examined to determine if there were changes in neuroanatomical structures. It was observed that serotonin levels rose in flies that were reared in the stimulus rich environment. It was also observed that over time the flies reared in the stimulus rich environment had a change in character of the neuroanatomical structure, the protocerebral bridge.

INTRODUCTION

Neurotransmitters are the signaling chemicals of our brain. They are responsible for the processing of information through the neural network of our brain and for regulating bodily functions. They are responsible, in part, for the formation of memory, adaptation to new environments and directing neuron growth and proliferation. Serotonin, 5-hydroxytryptamine, is a neurotransmitter that has been linked to such physiological functions as sleep, learning, behavior and digestion (Curzan 1978). Increased serotonin levels have been associated with a sense of well being and academic aptitude (Sylwester 1997). Low levels have been linked to aggression and

neuroses (Goldman 1996). Furthermore, rapid fluctuations in serotonin levels may lead to such pathological conditions as migraine headaches and neuralgia.

One factor that controls the level of serotonin in our system is our DNA. Genes code for the components of serotonin biosynthesis. Some allelic forms of these genes code for components that are more efficient at serotonin synthesis than other allelic forms (Goldman 1996). Another factor that influences serotonin levels is environmental stimulus. Those environments that provide sensory stimuli in abundance may cause serotonin levels to increase and also cause an increase neuron proliferation. The focus of my research is to investigate the relationship between environmental stimulus and serotonin production. I will also be investigating the relationship between environmental stimulus and neuron proliferation in the brain.

The model organism chosen was *Drosophila melanogaster*. There exists a wealth of information about the Drosophila genome. Previous studies have identified neuroanatomical structures and neurotransmitters involved in the information processing mechanism of this organism (Connolly et al. 1996, Davis 1996). The ability to cope with a sensory rich environment requires the integration of current sensory information with past environmental experience. In *D. melanogaster* this involves the neurotransmitter serotonin and the neuroanatomical structures mushroom bodies and the central body complex.

It is therefore suspected that flies reared in a stimulus rich environment will show an increase in both serotonin production and neuron proliferation.

MATERIALS AND METHODS

TREATMENT OF FLIES

D. melanogaster Oregon-R were reared in one of two environments for a predetermined length of time. One environment consisted of a single food source at a constant location, 25 ^{O}C and no light. The food source was a commercial yeast medium (Carolina Biological Supply). The

medium was replaced every five days under low light conditions. These conditions constituted the unchanging, deprived environment.

The second environment consisted of a 12 hours light, 12 hours dark cycle, ambient temperatures and several food choices which were changed every 24 hours both in content and location. The flies in this environment were given a commercial yeast medium plus a choice of bananas, grapes, watermelon, muskmelon, oranges, kiwi, mango, star fruit, pineapple and papaya. A different fruit was offered every 24 hours. These conditions constituted the enriched environment.

All flies used in this experiment were from the same laboratory stock. Adults were placed into vials containing commercial yeast medium and allowed to mate. When larvae were noticeable, the adults were released and the larvae were allowed to mature. From these vials, seventy-five age-matched flies were randomly selected, placed in a cage and put in the enriched environment. The same procedure was repeated for the deprived environment. Each cage was left in its respective environment for 5 days. This entire procedure was repeated for the 10 day and 15 day trials.

For the 0.5 day populations, the vials were established as before but as soon as larvae were noticeable and adults released, half the vials were placed in the dark and the other half were placed in a 12 hr light, 12 hr dark environment. Flies that eclosed in a 12 hour period were harvested.

ASSAY FOR SEROTONIN

Flies were sacrificed and the heads isolated at 0.5, 5, 10 & 15 days. The heads were all harvested at the same time of day to avoid fluctuations in serotonin levels due to circadian rhythms (Fowler et al. 1972). The heads were weighed and the weights recorded before transfer to a test tube containing 500µl 0.05M phosphate buffer. The heads were homogenized and

centrifuged. The supernatant obtained was frozen at -20 ^{O}C until serotonin levels could be determined.

Serotonin levels were determined using 20 µl of each sample supernatant. All samples were run in duplicate. The test used, enzyme linked immunosorbent assay (ELISA) produced a colored solution. The deeper the color, the less serotonin in the sample. The ELISA was based on a competitive inhibition binding of the antibody coated on to the wells. The antibody selectively binds any serotonin in the sample. Samples were incubated in the wells overnight, the plates were washed and a enzyme-conjugate was added. If the samples were high in serotonin there would be no binding sites left in the well. If , however, the samples were low in serotonin the enzyme-conjugate would bind to antibody still available in the well and when it did it would produce a colored solution. The level of serotonin was quantified by using a microplate reader that would determine the optic density (intensity of the color) of each solution. The concentrations were calculated by constructing a standard curve using known serotonin concentrations and plotting OD/ODmax. The OD/ODmax is taking the highest known concentration absorbance and establishing it as 100%. Then the other known samples were plotted as percentages of the highest absorbance. Therefore, unknown serotonin levels could be converted in a like manner and the concentrations determined. The average optical density of each sample was used to determine concentrations. All values of concentration were multiplied by 207.25 to take into account the dilution of the samples during the performance of the ELISA.

HISTOLOGICAL ASSAY

From each treatment group (0.5, 5, 10 & 15 days) fifty flies were processed for microscopic examination. These flies were randomly selected and transferred into a bath of 2.5% potassium dichromate. After 10 min they were transferred into vials containing 5 parts 2.5% potassium dichromate and 1 part 25% glutaraldehyde. The vials were kept in the dark at 4 ^{O}C for four days. The flies were then transferred into vials containing a 0.75% silver nitrate solution

where they remained for 48 hours. This treatment resulted in many individual neurons of the brain being impregnated with silver. These neurons appear black upon microscopic examination.

The heads were isolated, dehydrated and embedded in fresh paraffin. Serial sections of 10μm were mounted and checked for the level of silver impregnation. Those sections that showed little to no impregnation were stained with a hematoxylin and eosin series (H&E). This treatment would show neurons as "bubbly" structures and stain surrounding tissues pink. The sections were observed under oil immersion and the level of neuron proliferation was ascertained with special attention on the central body complex and mushroom bodies.

RESULTS

When gross head weight was compared, a difference between the enriched and deprived environments was observed. The flies reared in an enriched environment had an average head weight of 3.7 mg. The flies reared in the deprived environment had an average head weight of 2.8 mg. The biggest difference was observed in the 0.5 day populations. When just the 0.5 day population was compared, the flies that emerged in darkness had an average head weight of 2.3mg and those flies that emerged in a light/dark environment had an average head weight of 3.4mg (Fig. 1).

Serotonin concentrations were also observed to be different between the treatments. The highest concentrations of serotonin were observed in the 0.5 day and 10 day treatments (Fig. 3). There were noticeable differences between the two populations at 0.5 days and 10 days. There was a small difference observed between the two 5 day populations. The 15 day populations were not tested for serotonin concentrations.

Microscopic examination detected some difference in the degree of branching observed in the protocerebral bridge (a component of the central body complex) between the 15 day deprived flies and the 15 day stressful flies. The structures appeared larger on average in the stimulus rich reared flies than in the stimulus deprived flies(Fig.3).

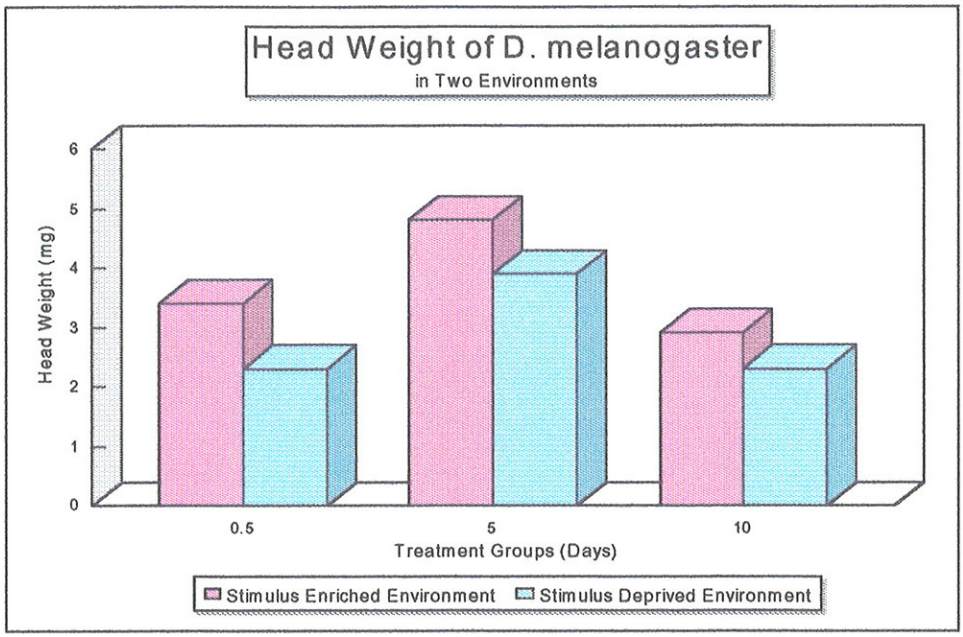

Fig.1 Head weight of *D. melanogaster* in two different environments.

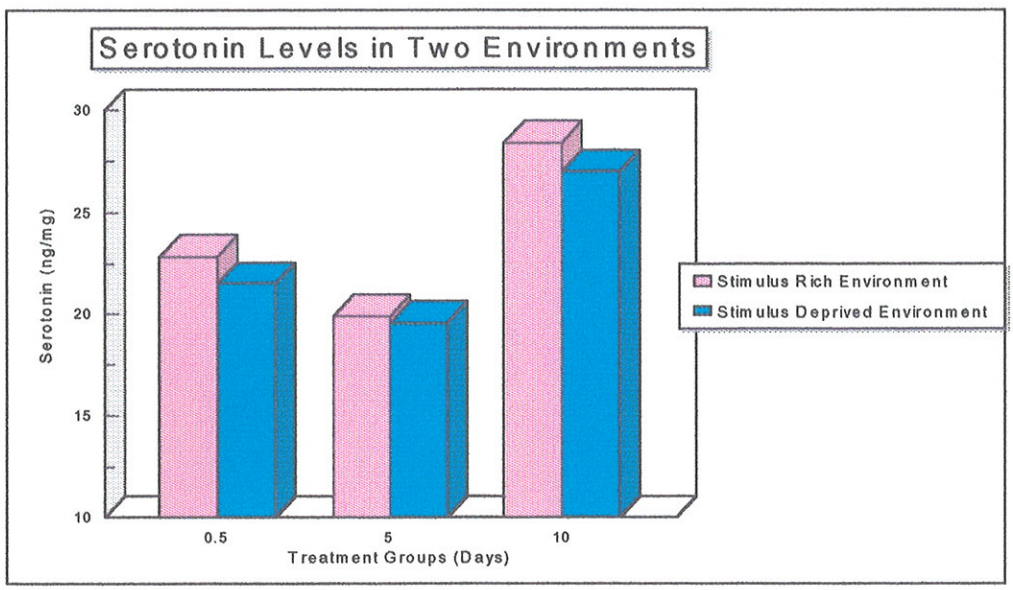

Fig 2 Serotonin concentrations in the heads of *D. melanogaster* reared in two different environments.

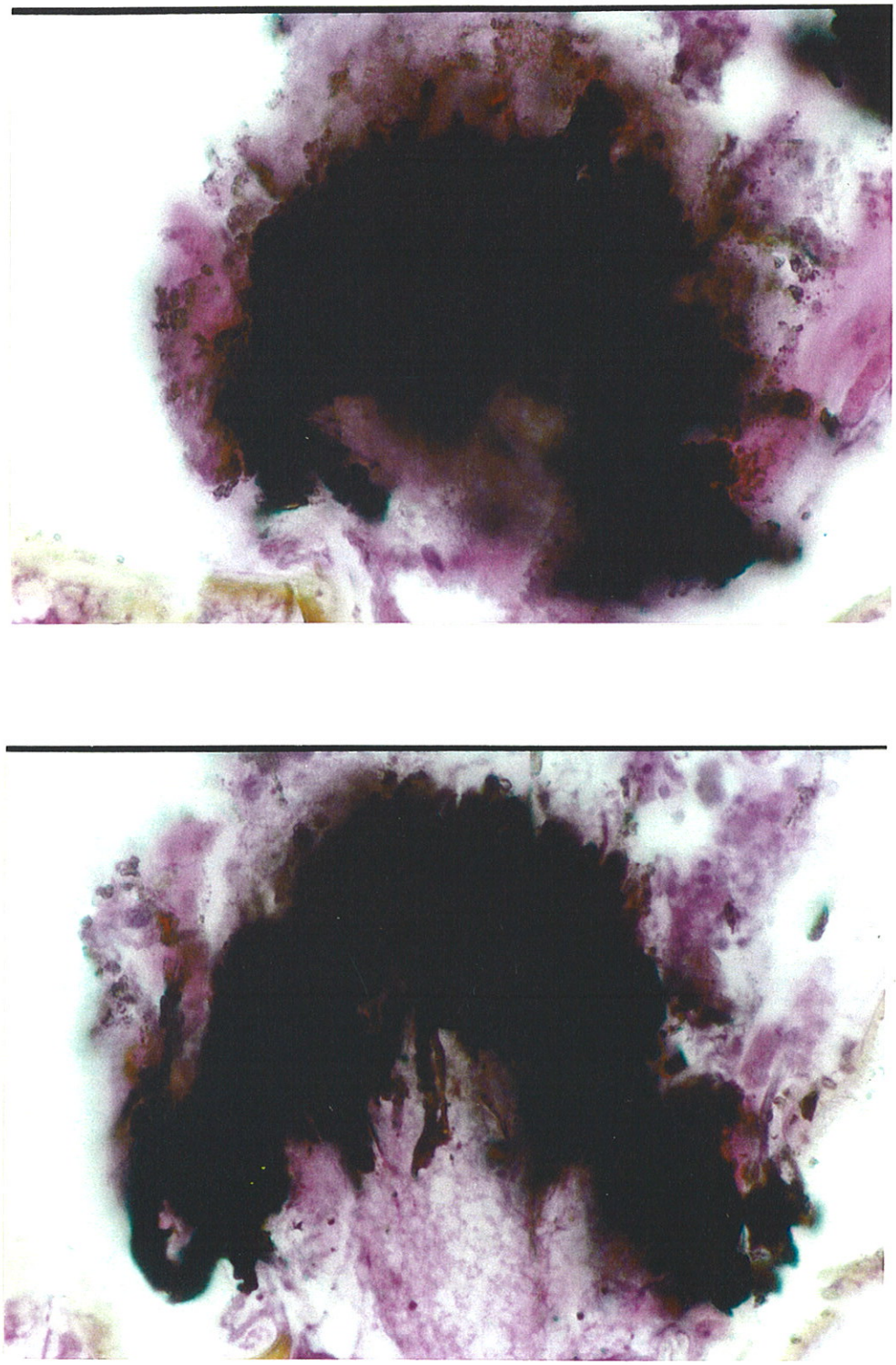

Fig. 3 15 Day enriched environment reared flies (top) and 15 Day Deprived environment reared flies (bottom). A difference in neuroanatomical character is evident.

DISCUSSION

The purpose of this research was to determine if environmental stimulus had an affect on serotonin production and neuron proliferation. The serotonin levels did rise in populations whose environment was stimulus rich. Serotonin production was detected in higher levels in the 0.5 day populations than in the 5 day populations. The levels rose again in the 10 day populations. This may indicate an age effect. *D. melanogaster* may produce higher levels of serotonin early in life to mediate the process of maturation. As flies age, the level of serotonin may drop to some basal level and then increase again in a response to the environment.

Head weight was also different between the two groups. This may be caused by the increase in protein production due to circadian rhythms in those flies that were exposed to a light stimulus. It is known that light stimulus triggers the downregulation of the timeless gene (*tim)* in *D. melanogaster* and there is growing evidence that the regulation of some biosynthetic pathways are controlled by the circadian rhythms of an organism (Hunter-Ensor et al. 1996) Furthermore, the products of the light regulated gene transcription may exist as hetereodimers during some portions of the light phase and as a monomer or not at all during the dark phase which might account for the increase in head weight.

Changes in the degree of neuron proliferation were not noticeable until the flies were 15 days of age. The protocerebral bridge, a component of the central body complex, exhibited a change in character in the enriched environment flies when compared to the deprived environment flies. This change in character may be an increase in neuron proliferation. However, this technique was not suitable for very young flies or for the definitive assessment of changes in neuron proliferation. To determine the nature of the character change a new technique will need to be employed.

The amount of stimulus in a given environment may have a direct effect on serotonin production and neuron proliferation in *D. melanogaster*. The level of serotonin production was observed to increase in *D. melanogaster* who were reared in a stimulus rich environment. A change in character of the neuroanatomical structures was also observed. The observed results could be in response to the circadian rhythms of the organism. Serotonin levels may remain low in the absence of light due to the regulatory action of the circadian rhythm. When light is present in the environment, the circadian rhythm may trigger the synthesis of serotonin and levels would be observed to increase.

FUTURE RESEARCH

In order to clarify the relationship of environmental factors and serotonin production more information is needed. My research suggests there may be a relationship between environmental changes, serotonin production and neuron proliferation. There are many studies which could be performed to clarify the nature of this relationship.

The first step would be to repeat this study over a longer period of time with numerous replicates of each population. Normative values for serotonin levels in *D. melanogaster* need to be established as well. Once the relationship has been demonstrated conclusively new questions can be asked about serotonin production in this organism. Furthermore, if serotonin production is in the control of the circadian rhythms then the changes in brain morphology may suggest the neuroanatomical structures involved with and serotonin synthesis and may provide clues about those structures involved in the circadian rhythm.

It would also be informative to rear flies in a deprived environment but not in the absence of light to ascertain if the circadian rhythm is the only mechanism controlling serotonin synthesis in these environments.

ACKNOWLEDGEMENTS

A special thanks to Dr. Charles Rodell for providing the fly stocks, Dr. Marcus Webster and Dr. Norman Ford for the purchase of the ELISA, Dr. Elizabeth Wurdak for histology instruction, Carol Jansky for help in procuring needed supplies, Dr. Steven Saupe for workspace, Dr. Ronald Henry for help with ELISA and Dr. Marilyn Meinke for manuscript review and experiment development.

REFERENCES

Connolly, J et al. "Associative learning disrupted by impaired G_s signaling in Drosophila mushroom bodies." Science. v.254 p. 2104 (4). 1996

Curzan G. "Serotonin and neurobiological disease" *In* Serotonin in Health and Disease. p.403 Essmann, W.B. ed. Spectrum Publications. New York. 1978

Davis R.L. "Physiology and biochemistry of Drosophila learning mutants." Physiological Reviews. v76. p 299 (19). April 1996.

Fowler, D., C. Goodnight and M. LaBrie. "Circadian rhythms of 5-hydroxytryptamine production in larvae, pupae and adults." The Annals of the Entomological Society. v 65 p. 138-141. 1972

Goldman, D. "High Anxiety. (genetic contributions to neuroticism)" Science. v.274 p.1483 Nov. 1996.

Hunter-Ensor M, A. Ousley & A. Sehgal. "Regulation of the Drosophila protein timeless suggests a mechanism for resetting the circadian clock." Cell. 84 p.677-685. 1996.

Sylwester, R. . "The neurobiology of self-esteem and aggression." Educational Leadership. v 54 p. 75-79. 1997